Transcriptional profiling reveals gene expression changes associated with inflammation and cell proliferation following short-term inhalation exposure to copper oxide nanoparticles

Supporting Information

Table S1. Real-time PCR analysis of selected markers in lung and liver.

Tissue	Gene	Concentration of CuO NPs (mg/m^3)	Fold Change	Significance
Lung	MIP-2α	0.6	-	-
		3.3	2.688	P<0.025
		13.2	3.718	P<0.01
	IL-1β	0.6	-	-
		3.3	3.013	P<0.025
		13.2	4.109	P<0.001
	TNFα	0.6	-	-
		3.3	-	-
		13.2	2.82	P<0.025
Liver	MT1A	0.6	-	-
		3.3	-1.9	P<0.025
		13.2	-	-

Animals were exposed to the 6 h equivalent exposure concentrations shown above and tissues were analysed on day 6, 1 day after the 5-day inhalation exposure. The fold change values represent the increase or decrease in gene expression that is both statistically significant and greater than the 1.6 fold change considered to be biologically significant.

Table S2. Protein expression in lung and liver tissue.

Tissue	Cytokine	Concentration of CuO NPs (mg/m^3)	Concentration of cytokine (pg/ml)	Significance
Lung	IL-1α	0.0	9.17±1.44	-
		0.6	31.47±6.08	P<0.001
		2.4	9.19±2.36	-
		3.3	25.16±2.27	P<0.01
		6.3	9.96±1.69	-
		13.2	23.51±1.78	P<0.01
	IL-1β	0.0	101.9±4.11	-
		0.6	274±67.6	P<0.01
		2.4	59.26±0.82	-
		3.3	191.1±25.6	-
		6.3	133.2±7.65	-
		13.2	260.2±19.7	P<0.01
	MIP-2α	0.0	3.44±0.31	-
		0.6	11.07±3.19	P<0.01
		2.4	2.79±0.93	-
		3.3	22.98±2.95	P<0.001
		6.3	4.87±1.2	-
		13.2	8.15±0.48	P<0.05
Liver	IL-1β	0.0	26.92±	-
		0.6	15.2±	-
		2.4	33.1±	-
		3.3	37.57±	-
		6.3	3.15±	P<0.05
		13.2	3.44±	P<0.05

Animals were exposed to the 6 h equivalent exposure concentrations shown above and tissues were analysed on day 6, 1 day after the 5-day inhalation exposure. The fold change values represent the increase or decrease in gene expression that is both statistically significant and greater than the 1.6 fold change considered to be biologically significant.

Fig. S1. Histological analysis. Sections of the lungs of rats (5 µm thick sections fixed in neutral-buffered formalin, tetrachrome stain using Alcian Blue and van Gieson's elastic stain) exposed to CuO NPs *via* inhalation for 5 consecutive days and analyzed at day-1 post-exposure and following a recovery period (at day-22 post-exposure). (A) Control animals, showing normal tissue. av) alveolus; bv) blood vessel; ep) epithelial cells; sp) alveolar septa. (B) Control animals, tissues collected after the recovery period. (C) and (D) rats exposed to the low dose. Note the presence of inflammatory cells in the first case (arrowheads) and hypertrophied epithelial cells (=ht). (E) Hyperplastic focus in the lungs of a rat exposed to the high dose at day-1 post-exposure. Note intrusion of inflammatory cells into alveoli and the thickening of septa caused by hypertrophy and hyperplasia of epithelial cells. *Inset*, low: evidence for necrotic macrophages. *Inset*, high: copper deposits inside a macrophage (arrowhead), detected using the Rubeanic Acid method. (F) Micrograph of the lung of a rat exposed to the high dose, collected at day-22 post-exposure (*i.e.*, after the recovery period). Scale bar: 25 µm.

Fig S2. DNA methylation analysis. Rats were exposed to 13.2 (HD) and 3.3 (LD) mg/m^3 CuO NPs for 5 days and samples were collected at day-1 post-exposure ('exposure') and at day-22 post-exposure ('recovery'). DNA methylation was assessed for a panel of known inflammation-related genes. (A) Heatmap linking the 22-gene list and experimental treatment (using Euclidean Distances and Pearson's *r* as metric). *Fadd* is highlighted with an (*). (B) Average methylation levels (% ± SD) for the *Fadd* gene. The different letters indicate significant differences (*t*-test, Bonferroni-corrected $p < 0.05$). (C) Average sum of methylation levels (% ± SD) combining all genes. No significant differences were found between experimental conditions.